# THE NATURE OF AN AMINO ACID RESIDUE AT THE ACTIVE CENTER OF FUMARASE[1]

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We are attempting to identify the amino acid residue(s) at the active center of fumarase by treating the enzyme with a reagent which has a specific affinity for this site and which may react irreversibly with it. ∝-Bromosuccinate closely resembles both malate (a natural substrate for fumarase) and succinate (a competitive inhibitor of fumarase) and should therefore bind at the enzyme's active center. In addition to this characteristic, -bromosuccinate should have the ability to alkylate the 1 (or 3) imidazole nitrogen in the histidine residue believed to be at the active center of fumarase (Alberty, 1961).

When heart muscle fumarase (Calbiochem) was incubated at 5° C for 10 minutes in a .01 M solution of ∝-bromosuccinate[2] at pH 7.3 complete inactivation of the enzyme was observed. In order to substantiate the view that ∝-bromosuccinate reacts irreversible with a histidine residue at the active center of fumarase and thereby inactivates the enzyme, we undertook the identification of 1 (or 3) histidine succinic acid (I) in the protein hydrolyzate of the inactivated fumarase.

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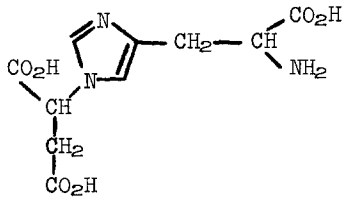
[2]K and K Chemical Co., Plainview, New York.

I 

Fumarase[3] (10 mg.) was placed in dialysis tubing and dialyzed against 25 ml. of a .02 M potassium phosphate buffer solution, pH 7.3, .01 M in α-bromosuccinate for 24 hours at 5° C. The inactivated enzyme was dialyzed against buffer to remove unreacted α-bromosuccinate and then repeatedly dialyzed against distilled water. Hydrolysis of the lyophilized inactivated enzyme in 2 ml. of 1:1 $H_2O$-concentrated HCl at 110° C for 22 hours in an evacuated sealed tube afforded the protein hydrolyzate used for amino acid analysis.

α-N-acetylhistidine succinic acid was prepared by dissolving α-N-acetylhistidine (100 mg., Calbiochem) and α-bromosuccinic acid (420 mg.) in 25 ml. of water, adjusting to the desired pH with NaOH, and incubating the solution at 50° C in a pH stat for 48 hours. A 50 ml. aliquot of the resulting solution was removed and hydrolyzed with 6 N HCl at 110° C in a sealed tube for 12 hours to yield I.

Amino acid analysis of the α-bromosuccinate inactivated fumarase (Fig. 1), carried out on a Beckman Model 120 B amino acid analyzer, showed a distinct peak just before aspartic acid and in exactly the same position where a sample of I was eluted from the 150 cm. column.

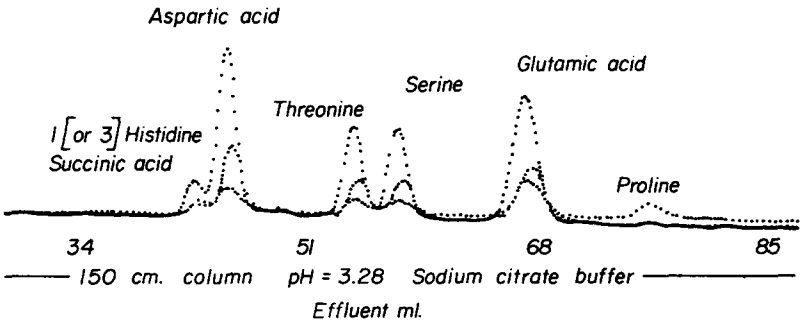



Fig. 1. A portion of the amino acid analysis of -bromosuccinate inactivated fumarase

[3]Heart muscle Fumarase (Calbiochem) was freed from the 2 M $(NH_4)_2SO_4$ packing solution prior to use by dialysis against .02 M potassium phosphate buffer solution for 48 hours.

It seems highly unlikely that α-bromosuccinate reacts indiscriminately with the histidine residues in fumarase, since the inactivation with α-bromosuccinate was carried out under extremely mild conditions, and since large amounts of unreacted histidine were observed in the amino acid analysis of the inactivated fumarase. Therefore, we conclude that α-bromosuccinate reacts selectively with a histidine residue at the active center of fumarase.

A quantitative determination of the number of alkylated histidine residues in α-bromosuccinate inactivated fumarase is now in progress.

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REFERENCES

Alberty, R. A., in The Enzymes, Vol. 5, Boyer, P. D., Lardy, H., and Myrbäck, K. (Editors), Academic Press, New York, 1961, p. 537.